

CLAIMS

1. A method for detecting and counting the microorganisms in a sample comprising the steps of:
  - 5 a) selectively enriching the microorganism sought in the sample,
  - b) conditioning of the aforementioned microorganism,
  - c) immunomagnetically concentrating the conditioned microorganism,
  - 10 d) fluorescently labeling the concentrated microorganism, and
  - e) detecting and analyzing the fluorescence.
2. A method according to claim 1, wherein the enrichment step is carried out in a composition comprising:
  - 15 - sodium pyruvate at a concentration ranging between 1 and 20 g/L, preferably between 1 and 10 g/L, and more preferably 4 to 6 g/L,
  - sodium thiosulfate at a concentration ranging between 0.5 and 5 g/L, preferably between 0.5 and 3 g/L, and still more preferably approximately 2 g/L,
  - 20 - catalase at a concentration ranging between 500 and 20 000 u/L, preferably between 2 000 and 8 000 u/L, and still more preferably approximately 5 000 u/L.
- 25 3. A method according to claim 2, wherein the aforementioned composition comprises in addition at least one antibiotic.
4. A method according to one of the claims 1 to 3, wherein the conditioning step is an induction step for at least one enzymatic activity specific to the microorganism sought, comprising adding to the microorganism's enrichment medium at least one non-fluorescent substrate specific to the aforementioned enzyme or enzymes.
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5. A method according to claim 4, wherein steps a) and b) can be carried out simultaneously.
6. A method according to claim 4 or 5, wherein step c) can take place before step b) or step c) can take place after step d).
7. A method according to one of the claims 1 to 3, wherein the conditioning step, in the case where the microorganism sought is a Gram-positive bacteria, comprises in addition an induction step for at least one surface antigen characteristic of the microorganism sought, comprising adding to the microorganism's enrichment medium yeast extract at a concentration ranging between 5 and 50 g/L, preferably between 10 and 20 g/L, and still more preferably approximately 10 g/L.
8. A method according to one of the claims 1 to 7, wherein the immunomagnetic concentration step comprises the steps of:
  - a) placing the microorganism sought, present in the conditioning medium, in contact with an antibody directed against an antigen specific to the microorganism, the aforementioned antibody being conjugated with a magnetic bead,
  - b) separating the bead-antibody-microorganism complexes from the medium,
  - c) separating the microorganism from the rest of the complex.
9. A method according to claim 8, wherein the antibody conjugated with a magnetic bead is directed against an antibody that is itself directed against an antigen specific to the microorganism sought.

10. A method according to claim 8 or 9, wherein the magnetic beads have a diameter ranging between 1 and 20  $\mu\text{m}$ , preferably between 2 and 8  $\mu\text{m}$ .
- 5 11. A method according to one of the claims 1 to 10, wherein fluorescent labeling of the microorganisms sought is carried out by adding to the medium containing the aforementioned microorganisms at least one substrate comprising a part specific to the enzymatic activity to be revealed and one label part.
- 10 12. A method according to claim 11, wherein the label part consists of a fluorogenic label excited at 488 nm chosen from the group comprising the xanthenes, acridines, phycobiliproteins, cyanine, and esculin.
- 15 13. A method according to claim 11 or 12, wherein the substrate part specific to the enzymatic activity to be revealed is chosen among a fatty acid, a monosaccharide, a phosphate, and/or a sulfate.
- 20 14. A method according to one of the claims 1 to 13, wherein the detection and analysis of fluorescence that make possible the numeration of the microorganisms are carried out by a technique chosen from the group comprising: flow cytometry, filtration cytometry and fluorescence microscopy.
- 25 15. A method according to one of the claims 1 to 14, wherein steps a), b), c), d), and e) as defined in claim 1 are preceded by a filtration step for the sample to be analyzed.
- 30 16. A method according to claim 15, wherein the filtration is carried out by means of a filter whose porosity ranges
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between 20 and 150 microns, preferably between 30 and 100 microns, and still more preferably approximately 63 microns.

- 5 17. A method according to claim 15, wherein the filtration is carried out on a membrane presenting a porosity ranging between 0.2 and 10  $\mu\text{m}$ , preferably between 0.2 and 5  $\mu\text{m}$ , and still more preferably between 0.2 and 0.5  $\mu\text{m}$ .
- 10 18. A selective enrichment medium for a microorganism sought in a sample comprising:
- a nutrient composition making the multiplication of the aforementioned microorganism possible, and
  - a selective revivification composition for the
- 15 the aforementioned microorganism, wherein it comprises:
- sodium pyruvate at a concentration ranging between 1 and 20 g/L, preferably between 1 and 10 g/L, and more preferably 4 to 6 g/L,
  - sodium thiosulfate at a concentration ranging between 0.5
- 20 and 5 g/L, preferably between 0.5 and 3 g/L, and still more preferably approximately 2 g/L,
  - catalase at a concentration ranging between 500 and 20 000 u/L, preferably between 2 000 and 8 000 u/L, and still more preferably approximately 5 000 u/L.

25 19. An enrichment medium according to claim 18, wherein it further comprises at least one antimicrobial agent.

30 20. A kit with which to implement the method of detecting and counting of a microorganism according to one of the claims 1 to 17, comprising:

  - an enrichment medium according to claim 18 or 19 in a liquid or dehydrated form, a plastic bag lined with a full-surface filter presenting a porosity of

35 approximately 63  $\mu\text{m}$ ,

- magnetic beads as defined in claim 8,
- one or several substrates as defined in claim 11 in a lyophilized form,
- appropriate solvents.